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Capsaicin cough sensitivity in smokers with and without airflow obstruction

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KEYWORDS
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Summary
Background: Cough is a frequent symptom of cigarette smokers that often precedes the development of airflow obstruction. We determined whether chronic cigarette smoking is associated with an increase in capsaicin cough response in the absence of cough.
Methods: We examined this in asymptomatic smokers with normal lung function ($n = 68$, FEV_1 $99.3 \pm 2.1\%$ predicted) and in patients with established COPD without cough symptoms ($n = 42$; FEV_1 $57.0 \pm 2.6\%$ predicted), using healthy non-smoking volunteers as control ($n = 92$; FEV_1 $100.6 \pm 1.7\%$ predicted). Using an incremental capsaicin concentration challenge protocol, we recorded the concentrations that induced 2 (C2) and 5 or more coughs (C5).
Results: Because females have a lower C2 and C5 than males in the control group, we analysed the data in each group according to gender. Log C5 was decreased both in asymptomatic smokers (1.56 ± 0.11 μmol/L, $p < 0.05$) and in COPD patients (1.44 ± 0.14 μmol/L, $p < 0.01$) when compared to non-smokers (1.90 ± 0.09 μmol/L). Log C2 did not differ between groups. Log C2 and log C5 were decreased in women (0.772 ± 0.071 μmol/L and 1.481 ± 0.094 μmol/L, respectively) when compared to men (1.045 ± 0.088 μmol/L and 1.923 ± 0.087 μmol/L, respectively) ($p < 0.05$ for log C2; $p < 0.001$ for log C5).
Conclusion: We conclude that chronic cigarette smoking increases capsaicin cough reflex and that this remains so with the development of COPD.

Abbreviations: COPD, chronic obstructive pulmonary disease; C2, concentration of capsaicin required to induce 2 coughs; C5, concentration of capsaicin required to induce 5 or more coughs; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

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Introduction

Cough is a frequent symptom of cigarette smokers and is often the first symptom of disease that precedes the development of airflow obstruction. Cough and sputum production for at least 3 months of the year in 2 consecutive years is used to define chronic bronchitis and are usually prominent features of early disease resulting from chronic cigarette smoking. There is now increasing evidence that chronic mucus hypersecretion may have an effect on decline in lung function.[1,2] The potential mechanisms of cough induced by cigarette smoking are numerous. Inflammatory mechanisms induced by constituents of cigarette smoke may lead to heightened cough reflex sensitivity, and excessive mucus production may directly induce cough. Cigarette smoke-exposed guinea-pigs develop an enhanced cough reflex to citric acid or capsaicin.[3]

In humans, cough responsiveness is usually studied by the inhalation of aerosols of irritant substances that stimulate cough, e.g. citric acid or capsaicin. Although these challenges have not gained widespread use in clinical monitoring, they are used as a research tool.[4] Using capsaicin cough challenge, an increased cough reflex was described in COPD patients as compared to a non-smoking normal population.[5] Paradoxically, in a small group of healthy cigarette smokers, a decrease in capsaicin cough reflex was reported, which was ascribed to increased mucus that would prevent stimulation of cough by restricting the access of capsaicin to the cough receptor.[6] Discrepancies exist between data of cough challenges obtained with citric acid and capsaicin in COPD patients in that there was a lower threshold to citric acid without concomitant change in capsaicin threshold.[7] One of the potential reasons for these discrepant results could be accounted for by the effect of airflow obstruction itself.

In the present study, we therefore tested the hypothesis that cigarette smoking enhances cough reflex sensitivity before having any significant impact on airflow obstruction. We used capsaicin challenge because this extract of red pepper induces cough in a dose-dependent and reproducible fashion and has over the years become the preferred agent for measurement of cough responsiveness thanks to its safety and minimal degree of tachyphylaxis.[8] In order to determine further the role of cough reflex in cigarette smoking, we examined capsaicin cough responsiveness in a cohort of 92 non-smoking and 110 smoking subjects of whom 68 had normal spirometric measurements and 42 had established COPD. Our data indicates that cough responsiveness is enhanced both in asymptomatic smokers with preserved lung function and in COPD patients with airflow obstruction.

Materials and methods

Study subjects

Asymptomatic adult smokers and healthy non-smokers not on any medication were recruited by advertisement. Adult smokers with COPD (defined as a FEV_1/FVC ratio <0.70) were recruited from the Royal Brompton Hospital clinics and were studied during a period of stability defined by no changes in symptoms for 4 weeks and no use of antibiotics or oral corticosteroids for 12 weeks. All smokers were smoking regularly at the time of study and none were on smoking cessation programmes or had tried to stop smoking in the past year. The non-smokers had never smoked in the past and were not exposed to secondary smoke. Most of the COPD patients were treated with short-acting bronchodilators and nearly half of them with inhaled corticosteroids and/or long-acting bronchodilators. Patients with a history of asthma were excluded. None of the patients or volunteers complained of a chronic cough. The study was approved by the Ethics Committee of the Royal Brompton and Harefield Trust and informed consent was obtained from each volunteer.

Measurements

Smokers were instructed not to smoke within 60 min of cough challenge and all subjects were asked not to take food or to do exercise also 60 min prior to challenge. No drinks containing caffeine were allowed for 4 h prior to cough challenge. Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were first measured in all subjects using a dry wedge spirometer (Vitalograph, Buckingham, UK).

Capsaicin cough challenge was performed by inhalation of incrementally doubling concentrations of capsaicin (0.9765–500 μmol/L), as previously described.[9] Capsaicin (8-Methyl-N-Vanillyl-6-Nonenamide; Sigma UK) was prepared as a 10^{-2} M stock solution in 100% ethanol stored at −20 °C. Fresh dilutions were made from this stock solution in 0.9% NaCl on a daily basis and stored at 4 °C. Two ml of solution were placed in a breath-activated nebuliser with a dosimeter (Mefar Dosimeter, Mefar, Italy) that was driven at a pressure of 151 kPa for a period of 1 s. Each puff delivered 20 μl, with a particle size of 4 μm mass median aerodynamic diameter at 1 min intervals. Patients were instructed to inspire from functional residual capacity to total lung capacity at a flow rate to trigger the dosimeter. They were asked to cough freely and the number of coughs induced during the 1 min period after capsaicin administration was counted, although most of the coughs occurred within 10 sof inhalation. The concentration of capsaicin required to induce 2 coughs (C2) or 5 coughs (C5) or more was recorded. The test was stopped when either C5 was obtained or when the highest capsaicin concentration (500 μmol/L) was reached without triggering more than 5 coughs. In the latter situation, the test was considered negative and an arbitrary value of 1000 was assigned. C2 and C5 values were converted to logC2 and logC5, respectively, for analysis.

Statistical analysis

Data are expressed as means ± SEM. Statistical analyses were performed using StatView version 5.0. Unpaired *t*-tests were used to analyse differences in quantitative data (age, log C2, log C5, FEV_1, FVC) between groups and the chi-square test was used to analyse dichotomous data. Correlation between two variables was calculated by linear

Table 1 Characteristics of normal volunteers and smokers.

	Non-smokers ($n = 92$)	Asymptomatic smokers ($n = 68$)	COPD patients ($n = 42$)
Male/female	43/49	30/38	22/20
Age, years (range)	34 ± 1 (21–87)	38 ± 2* (21–79)	64 ± 2** (37–84)
Height, cm	171.3 ± 0.9	169.4 ± 1.3	169.7 ± 1.4
Smoking status, pack years	0 ± 0	15.6 ± 1.8**	38.9 ± 3.1**
FEV_1 (L)	3.59 ± 0.09	3.35 ± 0.11	1.54 ± 0.09**
FEV_1 (% pred.)	100.6 ± 1.7	99. 3 ± 2.1	57.0 ± 2.6**
FVC (L)	4.22 ± 0.11	4.07 ± 0.13	2.77 ± 0.14**
FVC (% pred.)	100.8 ± 1.6	102.6 ± 2.1	82.9 ± 3.2**
FEV_1/FVC	85.2 ± 0.6	82.4 ± 0.8*	55.5 ± 1.5**

*: $p < 0.05$ *vs.* non-smokers. **: $p < 0.01$ *vs.* non-smokers. Values are means ± SEM.

regression using the least-squares method. $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of the patients are given in Table 1. Among COPD patients, 3 patients were classified as stage 1, 25 patients as stage 2, 12 patients as stage 3 and 2 patients as stage 4 according to the GOLD criteria.[10]

There were no unexpected adverse events reported. The capsaicin challenge was negative in 29 subjects (15 non-smokers, 10 asymptomatic smokers and 4 COPD). Compared to non-smokers, log C5 was lower in both asymptomatic smokers and in COPD patients (Fig. 1). However, there were no differences regarding the C2 values. The distribution of the log C5 values is shown in Fig. 2 with respect to FEV_1. There was no significant correlation between log C5 and either FEV_1 or FVC or FEV_1/FVC.

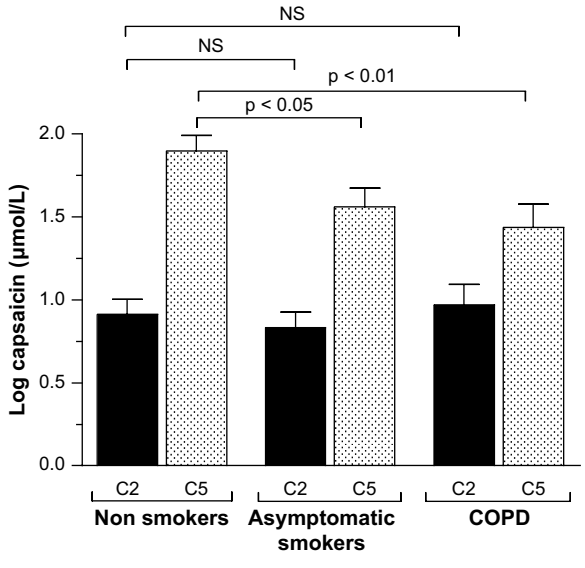

Figure 1 Log C2 and log C5 in non-smokers ($n = 92$), asymptomatic smokers ($n = 68$) and COPD patients ($n = 42$). Values are means ± SEM. C2: concentration of capsaicin required to induce 2 coughs. C5: concentration of capsaicin required to induce 5 or more coughs.

One factor influencing C5 (but not C2) was age. Log C5 was 1.806 ± 0.076 µmol/L in patients <50 years old *vs.* 1.403 ± 0.127 µmol/L in patients ≥50 years old ($p < 0.01$). Another factor influencing C5 as well as C2 was gender (Table 2). Log C2 was 0.772 ± 0.071 µmol/L in female *vs.* 1.045 ± 0.088 µmol/L in male ($p < 0.05$) while log C5 was 1.481 ± 0.094 µmol/L in female *vs.* 1.923 ± 0.087 µmol/L in male ($p < 0.001$).

Discussion

We have shown in this large cross-sectional study of chronic cigarette smokers that capsaicin cough sensitivity was increased in asymptomatic smokers compared to non-smoking controls in both male and female subjects. In comparison to non-smoking controls but not to asymptomatic smokers, there was an increase in capsaicin cough sensitivity in COPD patients, again shown in both genders. Therefore, capsaicin cough sensitivity increases with cigarette smoking irrespective of the development of COPD. We analysed our data according to gender since there is a known difference in capsaicin cough sensitivity with

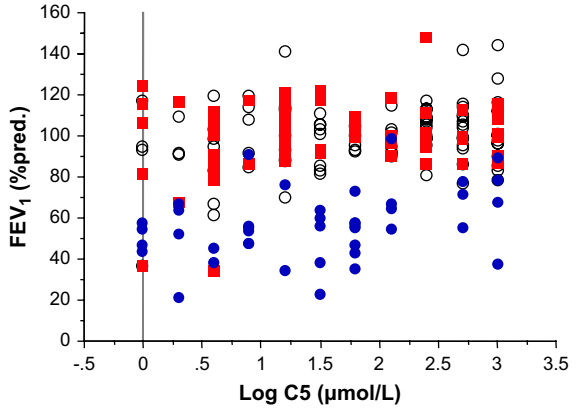

Figure 2 Relationship between FEV_1 (% predicted) and log C5 for non-smokers (○), asymptomatic smokers ■), and patients with COPD (●). There was no significant correlation. C5: concentration of capsaicin required to induce 5 or more coughs (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2 Gender-related differences in log C2 and log C5.

		Male	Female	*p* value
Non-smokers	*n*	43	49	
	Log C2	0.998 ± 0.124	0.844 ± 0.124	NS
	Log C5	2.111 ± 0.115	1.710 ± 0.144	0.04
Asymptomatic smokers	*n*	30	38	
	Log C2	1.013 ± 0.156	0.695 ± 0.105	0.08
	Log C5	1.796 ± 0.159	1.376 ± 0.159	0.07
COPD	*n*	22	20	
	Log C2	1.180 ± 0.203	0.742 ± 0.119	0.08
	Log C5	1.727 ± 0.204	1.119 ± 0.172	0.03

Values are means $\pm$ SEM.

women being more sensitive,[11,12] as we have confirmed with log C5 measurements. The differences in capsaicin cough sensitivity between men and women were maintained in all 3 groups studied, with women being more sensitive than men in all cases.

Our data are in agreement with those of Doherty et al.[5] who reported the presence of an increased cough reflex as measured by capsaicin responsiveness in patients with COPD, when compared to normal non-smoking subjects. The effect of smoking alone was not studied. We therefore extended these observations since we also found that capsaicin responsiveness was increased in smoking asymptomatic volunteers indicating that this increase was related to the effect of cigarette smoking rather than the development of COPD. Another important difference is that the asymptomatic smokers did not complain of cough despite an increase in capsaicin sensitivity, and it is possible that the degree of cough hypersensitivity was not large enough to lead to persistent cough. Similar to the findings of Doherty et al., we found no correlation between cough sensitivity and the degree of airflow obstruction in COPD as measured by FEV_1. Our results are on the other hand different from those of Wong and Morice[7] who reported no significant differences in capsaicin cough sensitivity in 16 COPD patients while citric acid cough sensitivity was increased. On the other hand, in 20 male current smokers, a diminution of capsaicin cough sensitivity compared to non-smoking control subjects has been reported.[6] Furthermore, the same group reported that cessation of smoking led to a reduction of capsaicin cough sensitivity.[13] Our data indicates that both male and female smokers maintain increased capsaicin cough sensitivity when compared to their respective controls, irrespective of gender. It remains difficult to reconcile these latter studies with ours and that of Doherty et al.[5] since all studies used similar methodologies for the capsaicin cough challenge.

We found that the C2 values did not show any differences between the groups, while the C5 values were consistently lower in the smoking groups that we studied. Another factor that relates to capsaicin cough responsiveness is age since we found that normal volunteers $>$50 years have a more sensitive cough reflex than those $<$50 years. This would suggest that the cough reflex increases with age, and may act as an improved protective reflex against aspiration which occurs more frequently in the elderly.

The increase in capsaicin cough responsiveness in cigarette smokers may relate to the degree of inflammatory response induced by chronic exposure to cigarette smoke. In guinea-pigs, exposure to cigarette smoke enhances capsaicin and citric acid cough responsiveness, an effect associated with an increase in the expression of CGRP-containing sensory nerves.[14] A dual neurokinin receptor antagonist that blocks both NK-1 and NK-2 receptors has been shown to inhibit the hypertussive citric acid response following cigarette smoking exposure in guineapigs.[3] Mutoh et al. showed that sensitisation of bronchopulmonary C-fibres endings by chronic exposure to side stream tobacco smoke was transmitted to the nucleus tractus solitarius and was associated with prolonged reflex evoked expiratory apnoea.[15] Further work by this group[16] showed that in young guinea-pigs exposed to environmental tobacco smoke, there was an increase in citric acid-induced cough through an NK-1 receptor mechanism in the brain stem nucleus tractus solitarius.

In summary, using an incremental capsaicin concentration challenge protocol, we found that the concentration that induced 5 or more coughs (C5) was decreased both in asymptomatic smokers and in COPD patients when compared to non-smokers. Cough reflex sensitivity assessed by C5 was affected by both age and gender. We suggest that an early effect of cigarette smoking is to increase capsaicin cough reflex sensitivity and that the development of COPD is not associated with a further increase in the cough reflex. However, longitudinal studies will be needed to confirm this hypothesis.

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Conflict of interest

The authors have no conflict of interest to disclose in relation to this work.

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